

Search for Genomic Alterations in Monozygotic Twins Discordant for Cleft Lip and/or Palate

Jane W. Kimani,^{1*} Koh-ichiro Yoshiura,² Min Shi,³ Astanand Jugessur,⁴ Danilo Moretti-Ferreira,⁵ Kaare Christensen,⁶ and Jeffrey C. Murray¹

¹ Department of Pediatrics, University of Iowa, United States of America

(*currently at Department of Pathology and Lab Medicine, University of North Carolina, United States of America)

² Department of Human Genetics, Nagasaki University Graduate School of Biomedical sciences, Nagasaki, Japan

³ Biostatistics Branch, National Institute of Environmental Health Sciences (NIEHS), United States of America

⁴ Craniofacial Development, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia

⁵ Servico de Aconselhamento, Genetico da Universidade Estadual Paulista, Botucatu, Brazil

⁶ Department of Epidemiology, Institute of Public Health, University of Southern Denmark, Denmark

Phenotypically discordant monozygotic twins offer the possibility of gene discovery through delineation of molecular abnormalities in one member of the twin pair. One proposed mechanism of discordance is postzygotically occurring genomic alterations resulting from mitotic recombination and other somatic changes. Detection of altered genomic fragments can reveal candidate gene loci that can be verified through additional analyses. We investigated this hypothesis using array comparative genomic hybridization; the 50K and 250K Affymetrix GeneChip® SNP arrays and an Illumina custom array consisting of 1,536 SNPs, to scan for genomic alterations in a sample of monozygotic twin pairs with discordant cleft lip and/or palate phenotypes. Paired analysis for deletions, amplifications and loss of heterozygosity, along with sequence verification of SNPs with discordant genotype calls did not reveal any genomic discordance between twin pairs in lymphocyte DNA samples. Our results demonstrate that postzygotic genomic alterations are not a common cause of monozygotic twin discordance for isolated cleft lip and/or palate. However, rare or balanced genomic alterations, tissue-specific events and small aberrations beyond the detection level of our experimental approach cannot be ruled out. The stability of genomes we observed in our study samples also suggests that detection of discordant events in other monozygotic twin pairs would be remarkable and of potential disease significance.

Keywords: monozygotic twins, discordant, cleft lip and palate, genome-wide

Classical twin research studies comparing disease concordance rates between monozygotic (MZ) and dizygotic (DZ) twins have been extensively applied to estimate the contribution of genetic and environmental factors to many complex traits (Boomsma et al., 2002). MZ twin concordance for common diseases and traits rarely reaches 100%, an observation that is

often attributed to differential environmental exposures (Wong et al., 2005). However, there are several molecular mechanisms that could underlie phenotypic discordance between MZ twins, such as *de novo* somatic mutations (Kondo et al., 2002), chromosomal anomalies (Gilbert et al., 2002), skewed X chromosome inactivation (De Gregorio et al., 2005), imprinting defects (Weksberg et al., 2002) and differential gene expression (Mak et al., 2004). The identification of molecular genetic differences between discordant MZ twins suggests that the utility of twin studies could be extended beyond heritability studies to gene discovery.

Postzygotic mitotic recombination and other somatic events such as deletions, nondisjunction, gene conversion, mobile genetic elements, fragile sites and repeat expansions have been proposed as a cause of MZ twin discordance (Cote & Gyftodimou, 1991; Kastern & Kryspin-Sorensen, 1988). Occurrence of somatic genomic changes in the early embryo can have significant implications for the phenotypes of the twins and the twinning process. If a genomic alteration occurs prior to twinning, unequal allocation of cells with the lesion to the two embryos could account for discordant phenotypes, with evidence of mosaicism, while the segregation of two genetically different populations of cells may trigger the twinning process, resulting in co-twins with discordant phenotypes (Machin, 1996). Since detection of chromosomal abnormalities relies on the comparison of a test genome with a reference genome sequence, the identical genomes of MZ twins ensure that any genomic differences can be established with confidence and

Received 11 November, 2008; accepted 17 June, 2009.

Address for correspondence: Jeffrey C. Murray, MD, University of Iowa, Department of Pediatrics, S. Grand Avenue, 2182 ML, Iowa City IA 52242 USA. E-mail: jeff-murray@uiowa.edu

accuracy. This approach is particularly advantageous for delineating disease causing copy number variants that could arise in one member of the twin pair. Any genomic mismatches between the twins' genomes could be revealed as amplifications, deletions or loss of heterozygosity. Recent identification of novel cleft lip and/or palate (CLP) candidate genes by genome-wide array-CGH highlights the impact of genomic alterations in the etiology of this complex disorder (Osoegawa et al., 2008).

CLP occurs in approximately 1 out of 700 births worldwide and has a significant clinical and economic impact. Approximately 40% of MZ twins are discordant for the disease phenotype (Christensen & Fogh-Andersen, 1993a). Genetic factors contributing to disease etiology include *IRF6* (Zuccherro et al., 2004); *MSX1* (Jezewski et al., 2003); *RYK* (Watanabe et al., 2006) and genes in the *FGF* pathway (Riley et al., 2007). Additionally, nongenetic factors such as maternal cigarette smoking and nutrition also play a role in CLP etiology (Jugessur & Murray, 2005).

The complex nature of CLP complicates traditional mapping approaches such as linkage and association,

which rely on population-matched or family-based controls to determine if a sequence variant is disease-causing. Based on identical genetic backgrounds, the unaffected twin in a discordant pair provides a well-matched control for studying a complex disease such as CLP thereby eliminating the need for external controls. We have previously reported our model using MZ twins discordant for CLP in gene discovery (Mansilla et al., 2005). In this report, we describe our search for genomic alterations in MZ twins discordant for CLP using array CGH and high-density SNP genotyping arrays.

Materials and Methods

Study Subjects

A summary of the discordant MZ twin samples and the respective analyses employed for each twin pair is presented in Table 1. The variability in the methods used to analyze the different samples resulted from multiple stages of the study being conducted over a period of time. All affected individuals were nonsyndromic cases of CLP. We used DNA previously

Table 1

Twin Samples Analyzed in This Study

Twin pair no.	Twin sample data			Analysis method			
	Country	Sex	Phenotype	Array CGH	Affymetrix 50K	Affymetrix 250K	Custom Illumina SNPs
1	P	F	CL+P	✓			✓
2	P	M	CLO	✓	✓		✓
3	P	M	CL+P	✓			✓
4	P	F	CLO	✓		✓	✓
5	P	F	CL+P	✓	✓		✓
6	C	M	CL+P	✓		✓	
7	C	M	CLO			✓	
8	P	F	CL+P			✓	✓
9	B	M	CL+P			✓	
10	B	F	CLO			✓	✓
11	B	M	CLO			✓	✓
12	B	F	CLO			✓	
13*	B	F	CL+P			✓	
14	P	M	CL+P			✓	✓
15	A	F	U/T				✓
16	A	M	U/T				✓
17	A	M	U/T				✓
18	D	M	CLO				✓
19	D	M	CLO				✓
20	D	F	CLO				✓
21	D	F	CLO				✓
22	D	F	CLO				✓
23	D	M	CLO				✓
24	D	F	CL+P				✓
25**	D	F	CPO				✓

Note: *Father's DNA sample unavailable; **Triplets; A-Australia; B-Brazil; C-Colombia; D-Denmark; P-Philippines; U/T-Untyped cleft phenotype.

extracted from peripheral blood lymphocytes. Genotyping a set of DNA markers had previously established that the twins were MZ. Informed consent was obtained for all study participants and the institutional review board approved the study.

Array CGH

CGH is a molecular cytogenetic technique for analyzing DNA copy number variations. DNA from a test and reference sample are differentially labeled and hybridized to an array spotted with a genomic representation that allows detection of copy number differences between the two samples at specific genomic locations (Pinkel & Albertson, 2005). For our analysis, Koh-ichiro Yoshiura et al. at Nagasaki University developed an array spotted with 2,173 genomic BAC clones. Samples from six pairs of twins were analyzed: for each pair, the DNA samples were differentially labeled, hybridized, and then scanned. Reverse sample labeling was also performed. The average normalized inter-locus fluorescence ratio (ANILFR) between the affected and unaffected twin samples was calculated. Prior preliminary experiments using five sets of normal/normal control samples had established the thresholds for copy number gain and loss at 0.86 and 1.18 with a standard deviation (SD) of 0.06, so the normal ANILFR range was defined as within ± 2 SD.

SNP Genotyping Arrays

Large scale SNP genotyping allows detection of allelic imbalances such as loss of heterozygosity (LOH) and copy number changes through hybridization signal intensities. In our analysis, we utilized a custom BeadArray™ platform from Illumina, Inc., (San Diego, CA, USA) and Affymetrix GeneChip® Human Mapping 50K and 250K sets (Santa Clara, CA, USA).

Illumina custom SNP genotyping. The Illumina BeadChip is a platform for performing multiplex gene analyses using oligonucleotides attached to silica beads. Defined SNPs can be chosen for a custom genotyping array based on the interests of the researcher (Steemers & Gunderson, 2007). For our analysis, we

selected 1,536 SNPs representing 388 CLP candidate genes for a single genotyping array. The experiments were carried out at the Center for Inherited Disease Research (CIDR) according to the manufacturer's instructions and included 20 pairs of discordant MZ twins. Data were analyzed using Illumina's BeadStudio v2 genotyping and LOH plus module software that allow detection of chromosomal aberrations and allelic imbalance in paired samples (www.illumina.com).

Affymetrix SNP genotyping. Affymetrix GeneChips employ a whole genome sampling analysis method to genotype thousands of SNPs on synthetic oligonucleotide arrays by allele-specific hybridization. DNA samples were prepared for analysis and hybridization according to the manufacturer's instructions (www.affymetrix.com). We used the GeneChip® Human Mapping 50K Xba I ($n = 2$ twin pairs) and the 250K Nsp I ($n = 10$ twin pairs) arrays. SNP allele calls were assigned using a Dynamic Model mapping algorithm, a highly accurate genotype calling method that uses a one-sided Wilcoxon signed rank test to provide a confidence score (CS) for each genotype. The CS measures the reliability of a genotype call thus filtering out SNPs with a high error rate as 'no-calls' (Matsuzaki et al., 2004). Gender status for each sample is inferred based on X chromosome heterozygosity. We performed a paired analysis for LOH and copy number changes using the Affymetrix Chromosome Copy Number Analysis Tool 4.0 (CNAT 4.0).

DNA Sequencing

Genotypes generated using the Affymetrix 50K and 250K GeneChips that had discordant allele calls and a confidence score of ≤ 0.05 were identified as candidates for DNA sequencing to verify the SNP genotypes. After ranking by the significant confidence scores, at least 10 SNPs that did not fall within repeat elements were selected for sequencing in each twin pair. DNA samples from the parents were included in the sequencing analysis to verify Mendelian segregation of alleles. PCR reactions were performed on Applied Biosystems Gene Amp PCR System 9700 with 20ng

Table 2

BAC Clones With Significantly High Signal Differences From aCGH

BAC Clone	Locus	Twin pair no.					
		1 ^a	2 ^b	3 ^a	4 ^{b,c}	5 ^{b,b}	6 ^c
GS-98C4	XpYpter			✓	✓	✓	
RP11-89P7	2q32.1 - 2q32.2			✓		✓	
RP11-1145M16	7q11.2					✓	✓
RP11-117N14	8q21.11	✓			✓		
RP11-613G2	11p15.5		✓				✓
RP11-75H6	19p13.3			✓			✓
RP11-17K15	19p13.3		✓			✓	
RP11-197O4	19p13.2			✓	✓		

Note: Samples also genotyped with the Illumina genotyping panel^a, the 50K Affymetrix GeneChip^b, or the 250K Affymetrix GeneChip^c.

Table 3

Summary of Affymetrix GeneChips Genotyping Results

50K Affymetrix GeneChip				
Twin pair no.	SNP Call rate (affected/unaffected) average: 91.4%	Genotype concordance average -99.7%	No. of discordant genotypes	Discordant genotypes with ≤ 0.05 confidence score
2	90.7 / 92.7	99.7	180	4
5	91.3 / 91.5	99.8	137	5
250K Affymetrix GeneChip				
Twin pair no.	SNP Call rate Average -91.5%	Genotype concordance Average -98.8%	No. of discordant genotypes	Discordant genotypes with ≤ 0.05 confidence score
4	95.7 / 93.9	99.3	1818	13
6	87.7 / 88.3	98.6	3668	16
7	86.0 / 85.7	98.0	5349	64
8	93.4 / 94.0	99.2	2126	7
9	90.8 / 90.7	98.6	3607	32
10	91.9 / 92.1	98.9	2945	27
11	95.3 / 93.6	99.2	2027	15
12	94.5 / 92.4	99.0	2528	24
13	93.2 / 93.0	99.0	2711	35
14	92.6 / 90.5	98.8	3052	35

DNA and 0.25 units of Biolase (Bioline, Randolph, MA). Sequencing was carried out with the Big Dye™ Terminator cycle sequencing and run on ABI Prism 3730 DNA Analyzer (Applied Biosystems). Sequences were assembled using the Phred-Phrap package and visualized using the Consed program.

Results

Array CGH Results

With 2,173 BAC clones spread across the genome, the aCGH provided a resolution of approximately 1-Mb. Table 2 shows 8 BAC clones with fluorescence ratios that deviated significantly (> 3 SD) in at least two twin pairs. The regions covered by these BACs do not overlap with any known CLP candidate loci, nor contain genes showing high craniofacial expression according to COGENE — the Craniofacial and Oral Gene Expression Network. Genomic region 19p13 looked especially interesting with 3 BACs showing high signal differences, so we genotyped two SNPs of high heterozygosity within each BAC in the twins and the parents, but did not find evidence for allelic imbalance.

Illumina Genotyping Results

The average SNP call rate on the Illumina genotyping panel was approximately 89%. We did not observe any discordant genotypes between pairs of MZ twins. A paired analysis with the Beadstudio software was performed for LOH and copy number changes at a 1 Mb window size. Although the sparse SNP coverage of ~1,500 SNPs greatly limited the power of this analysis, there was no indication of LOH in the twin pairs. A few genomic regions revealed possible copy

number changes for twin pairs 3–6, but the more comprehensive genotype data generated from Affymetrix arrays disproved that observation.

Affymetrix Genotyping Results

Genotyping data and concordance rates for the samples scanned with the Affymetrix GeneChips are summarized in Table 3. The call rate from 58,960 and 262,264 SNPs for the 50K and 250K GeneChips respectively was $> 90\%$ for all samples. The average proportion of genotypes that were concordant between twin pairs was ~99% for both GeneChips. This was comparable to the 98% degree of genotype concordance observed in comparing two independent 250K array scans of the unaffected individual in twin pair no. 6, which revealed high reproducibility of the genotype calls.

Sequencing Results

DNA sequencing was carried out for a total of 107 regions surrounding SNPs that had received discordant genotype calls from the Affymetrix GeneChip analyses within twin pairs. Results revealed 181 SNP genotypes that were concordant between twin pairs (Table 4). Additionally, sequencing of DNA samples from the parents showed consistency with Mendelian inheritance.

Discussion

The advent of genome scanning tools allows comprehensive analysis of chromosomal rearrangements, dependent on the resolution of the experimental approach. The aCGH experiment using genomic

Table 4

Summary of SNPs Genotyped by Sequencing to Confirm Concordance Between Twin Pairs

Twin pair		Region (no. SNPs)												
2	2q23.3 (1)	5q12 (1)	10q21 (1)	16p12.1 (1)										
5	8q24.12 (3)	10q21.1 (1)	12q13.13 (1)	15q26.2 (1)										
4	2q14.3 (1)	3q27.2 (1)	10p15.2 (1)	10q26.2 (1)	13q31.1 (1)	16q23.3 (1)	21q21.1 (2)							
6	1q32.1 (4)	4p15.32 (3)	4q32.3 (1)	5q21.3 (1)	7p12.1 (3)	7q31.32 (2)	8q22.1 (2)	9p24.2 (2)	10q11.23 (1)	10q21.1 (4)	11q24.2 (1)	12q15 (1)	14q31.3 (1)	
7	2q21.3 (1)	5p15.33 (2)	6q14.1 (1)	7p21.1 (1)	12q21.2 (1)	12q21.32 (2)	12q23.2 (2)	16q22.3 (1)	18q12.3 (1)	19q13.32 (4)				
8	2p24.1 (1)	2q21.3 (1)	4p13 (1)	5q21.3 (1)	8q24.22 (3)	11p13 (2)	12q21.33 (1)	21q21.1 (2)	Xq25 (2)					
9	2q13 (2)	2q31.2 (2)	4q31.23 (1)	5p13.3 (1)	5q14.1 (3)	7q21.11 (1)	10q23.31 (3)	11p15.4 (2)	11p14.2 (2)	17q11.2 (1)				
10	1q32.1 (4)	1q32.1 (5)	2p16.1 (3)	3p14.2 (1)	5p13.2 (1)	5q14.3 (3)	5q35.2 (3)	7p21.2 (1)	8q23.2 (1)	14q21.3 (2)				
11	1q25.3 (1)	2q34 (1)	4q22.3 (3)	5q12.1 (3)	7p21.2 (1)	7q21.3 (2)	11p14.1 (1)	12p12.3 (1)	12q12 (1)	Xq28 (2)				
12	1p36.12 (1)	1q44 (3)	2p24.3 (5)	4p16.3 (1)	6q13 (3)	6q16.1 (1)	7p15.1 (1)	10q21.3 (1)	10q26.11 (1)	18q12.3 (1)				
13	1p12 (1)	1q24.2 (4)	3q23 (2)	3q25.33 (1)	6p25.3 (1)	6q16.3 (2)	9p21.3 (1)	10p15.1 (1)	16q12.2 (2)	17q22 (1)				
14	3p12.3 (1)	3q13.33 (1)	4p12 (2)	6q16.1 (1)	8p23.2 (2)	8q23.1 (2)	8q23.2 (1)	10p12.32 (1)	10q21.3 (2)	11p11.2 (1)				

BACs provided extensive coverage of the genome at a resolution of ~1 Mb, so any smaller chromosomal aberrations would not be detected. SNP arrays not only provide genotypes for thousands of SNPs, but can also be used to detect copy number changes based on hybridization signal intensities. Genotype data is useful for detecting loss of heterozygosity in chromosomal regions with deletions or uniparental disomy. At an average inter-marker distance of ~60 kb and ~12 kb for the 50K and 250K GeneChips respectively, and additional genotyping through the Illumina SNP panel, our analysis provide sufficient resolution to detect submicroscopic structural variants that are defined in the range of ~10 kb to 3 Mb (Feuk et al., 2006). Several samples were interrogated using more than one method (table 1), thus allowing data comparison and verification.

Our study using aCGH and genotyping arrays did not reveal any genomic alterations within MZ twins discordant for nonsyndromic CLP. Genomic alterations could be confined to specific tissues depending on the timing of the mutational event, resulting in mosaicism. Since acquisition of DNA samples from lip and palatal tissues is difficult, our analysis was limited to analysis of DNA samples collected from peripheral blood lymphocytes, which may not accurately represent the target tissues affected in CLP. Additionally, our experimental approaches were incapable of detecting balanced variants such as those resulting from inversions and translocations, and chromosomal aber-

rations that involve regions with only homozygous alleles. Routine karyotyping which can generally detect such chromosomal abnormalities could not be performed due to unavailability of living cells from a blood sample.

We are unaware of definitive reports of postzygotic genomic rearrangements underlying MZ twin discordance. A recent report of copy number variants arising between MZ twins during somatic development is consistent with our rationale for using discordant MZ twins in disease gene identification (Bruder et al., 2008). Changes in the somatic genome are well recognized as a source of diversity within the immunoglobulin and T-receptor genes (Kastern & Kryspin-Sorensen, 1988). In disease states, rearrangements can induce a phenotype by directly interrupting a gene sequence, altering gene dosage, or gene expression through position effects (Lupski & Stankiewicz, 2005). Mitotic recombination is especially relevant in tumor development, since it can lead to the expression of recessive tumor suppressor genes and/or amplification of protooncogenes (Gupta et al., 1997). Analysis of MZ twins concordant for cancer can reveal DNA rearrangements that are common to both twins as potential candidates for susceptibility loci (el-Rifai et al., 1999). In addition to oncogenesis, somatic mutations mediated through LOH can potentially contribute to other biological processes such as aging (Grist et al., 1992).

Phenotypic discordance is a common observation in complex genetic diseases, and a postzygotic somatic change is only one of several proposed mechanisms for discordance. Since CLP is a congenital defect, non-genetic intrauterine environmental factors such as unequal cell allocation at twinning and disproportionate placental blood supply may contribute to discordance (Gringras & Chen, 2001). If such developmental influences alone were responsible for MZ twin discordance, then one would expect an excess of CLP in MZ twins compared to singletons. This is not the case as studies in Denmark have demonstrated no significant difference in prevalence of CLP in twins versus singletons (Christensen & Fogh-Andersen, 1993a; 1993b). The absence of genomic differences between MZ twins underscores their use as matched case-controls particularly in studies exploring the environmental component in complex traits. With the availability of array-based techniques to simultaneously scan the whole genome, it is now possible to map genomic alterations at a high resolution and additional experimental tools such as quantitative PCR and multiplex ligation-dependent probe amplification (MLPA) can be applied to independently confirm any observed genomic alterations. This straightforward approach can be applied to other cases of discordant MZ twins where identification of genomic alterations can reveal potential candidate genes, or provide evidence of a gene's involvement in disease etiology.

Acknowledgments

We would like to thank the patients and families who contributed samples and information for this study. We are also grateful to Marla Johnson for technical assistance. This study was supported by the following grants: R37DE008559, P50DE016215, R01DE015197, and by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (Z01-ES04007-12).

References

- Boomsma, D., Busjahn, A., & Peltonen, L. (2002). Classical twin studies and beyond. *Nature Review Genetics*, 3, 872–882.
- Bruder, C. E., Piotrowski, A., Gijsbers, A. A., Andersson, R., Erickson, S., de Stahl, T. D., Menzel, U., Sandgren, J., von Tell, D., Poplawski, A., Crowley, M., Crasto, C., Partridge, E. C., Tiwari, H., Allison, D. B., Komorowski, J., van Ommen, G. J., Boomsma, D. I., Pedersen, N. L., den Dunnen, J. T., Wirdefeldt, K., & Dumanski, J. P. (2008). Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. *American Journal of Human Genetics*, 82, 763–771.
- Christensen, K., & Fogh-Andersen, P. (1993a). Cleft lip (+/- cleft palate) in Danish twins, 1970–1990. *American Journal of Medical Genetics*, 47, 910–916.
- Christensen, K., & Fogh-Andersen, P. (1993b). Isolated cleft palate in Danish multiple births, 1970–1990. *The Cleft Palate-craniofacial Journal*, 30, 469–474.
- Cote, G. B., & Gyftodimou, J. (1991). Twinning and mitotic crossing-over: Some possibilities and their implications. *American Journal of Human Genetics*, 49, 120–130.
- De Gregorio, L., Jinnah, H. A., Harris, J. C., Nyhan, W. L., Schretlen, D. J., Trombley, L. M., & O'Neill, J. P. (2005). Lesch-Nyhan disease in a female with a clinically normal monozygotic twin. *Molecular Genetics and Metabolism*, 85, 70–77.
- el-Rifai, W., Tarmo, L., Hemmer, S., Forsti, A., Pedersen, N., Lichtenstein, P., Ahlbom, A., Soderberg, M., Knuutila, S., & Hemminki, K. (1999). DNA copy number losses at 1p32-pter in monozygotic twins concordant for breast cancer. *Cancer Genetics and Cytogenetics*, 112, 169–172.
- Feuk, L., Carson, A. R., & Scherer, S. W. (2006). Structural variation in the human genome. *Nature Review Genetics*, 7, 85–97.
- Gilbert, B., Yardin, C., Briault, S., Belin, V., Lienhardt, A., Aubard, Y., Battin, J., Servaud, M., Philippe, H. J., & Lacombe, D. (2002). Prenatal diagnosis of female monozygotic twins discordant for Turner syndrome: implications for prenatal genetic counselling. *Prenatal Diagnosis*, 22, 697–702.
- Gringras, P., & Chen, W. (2001). Mechanisms for differences in monozygous twins. *Early Human Development*, 64, 105–117.
- Grist, S. A., McCarron, M., Kutlaca, A., Turner, D. R., & Morley, A. A. (1992). In vivo human somatic mutation: frequency and spectrum with age. *Mutation Research*, 266, 189–196.
- Gupta, P. K., Sahota, A., Boyadjiev, S. A., Bye, S., Shao, C., O'Neill, J. P., Hunter, T. C., Albertini, R. J., Stambrook, P. J., & Tischfield, J. A. (1997). High frequency in vivo loss of heterozygosity is primarily a consequence of mitotic recombination. *Cancer Research*, 57, 1188–1193.
- Jezewski, P. A., Vieira, A. R., Nishimura, C., Ludwig, B., Johnson, M., O'Brien, S. E., Daack-Hirsch, S., Schultz, R. E., Weber, A., Nepomucena, B., Romitti, P. A., Christensen, K., Orioli, I. M., Castilla, E. E., Machida, J., Natsume, N., & Murray, J. C. (2003). Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. *Journal of Medical Genetics*, 40, 399–407.
- Jugessur, A., & Murray, J. C. (2005). Orofacial clefting: recent insights into a complex trait. *Current Opinion in Genetics & Development*, 15, 270–278.
- Kastern, W., & Kryspin-Sorensen, I. (1988). Penetrance and low concordance in monozygotic twins in disease: are they the results of alterations in somatic genomes? *Molecular Reproduction and Development*, 1, 63–75.
- Kondo, S., Schutte, B. C., Richardson, R. J., Bjork, B. C., Knight, A. S., Watanabe, Y., Howard, E., de Lima, R.

- L., Daack-Hirsch, S., Sander, A., McDonald-McGinn, D. M., Zackai, E. H., Lammer, E. J., Aylsworth, A. S., Ardinger, H. H., Lidral, A. C., Pober, B. R., Moreno, L., Arcos-Burgos, M., Valencia, C., Houdayer, C., Bahuau, M., Moretti-Ferreira, D., Richieri-Costa, A., Dixon, M. J., & Murray, J. C. (2002). Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nature Genetics*, 32, 285–289.
- Lupski, J. R., & Stankiewicz, P. (2005). Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genetics*, 1, e49.
- Machin, G. A. (1996). Some causes of genotypic and phenotypic discordance in monozygotic twin pairs. *American Journal of Medical Genetics*, 61, 216–228.
- Mak, Y. T., Hampson, G., Beresford, J. N., & Spector, T. D. (2004). Variations in genome-wide gene expression in identical twins: A study of primary osteoblast-like culture from female twins discordant for osteoporosis. *BMC Genetics*, 5, 14.
- Mansilla, M. A., Kimani, J., Mitchell, L. E., Christensen, K., Boomsma, D. I., Daack-Hirsch, S., Nepomucena, B., Wyszynski, D. F., Felix, T. M., Martin, N. G., & Murray, J. C. (2005). Discordant MZ twins with cleft lip and palate: A model for identifying genes in complex traits. *Twin Research and Human Genetics*, 8, 39–46.
- Matsuzaki, H., Dong, S., Loi, H., Di, X., Liu, G., Hubbell, E., Law, J., Berntsen, T., Chadha, M., Hui, H., Yang, G., Kennedy, G. C., Webster, T. A., Cawley, S., Walsh, P. S., Jones, K. W., Fodor, S. P., & Mei, R. (2004). Genotyping over 100,000 SNPs on a pair of oligonucleotide arrays. *Nature Methods*, 1, 109–111.
- Osoegawa, K., Vessere, G. M., Utami, K. H., Mansilla, M. A., Johnson, M. K., Riley, B. M., L'Heureux, J., Pfundt, R., Staaf, J., van der Vliet, W. A., Lidral, A. C., Schoenmakers, E. F., Borg, A., Schutte, B. C., Lammer, E. J., Murray, J. C., & de Jong, P. J. (2008). Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridisation. *Journal of Medical Genetics*, 45, 81–86.
- Pinkel, D., & Albertson, D. G. (2005). Comparative genomic hybridization. *Annual Review of Genomics and Human Genetics*, 6, 331–354.
- Riley, B. M., Mansilla, M. A., Ma, J., Daack-Hirsch, S., Maher, B. S., Raffensperger, L. M., Russo, E. T., Vieira, A. R., Dode, C., Mohammadi, M., Marazita, M. L., & Murray, J. C. (2007). Impaired FGF signaling contributes to cleft lip and palate. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 4512–4517.
- Steemers, F. J., & Gunderson, K. L. (2007). Whole genome genotyping technologies on the BeadArray platform. *Biotechnology Journal*, 2, 41–49.
- Watanabe, A., Akita, S., Tin, N. T., Natsume, N., Nakano, Y., Niikawa, N., Uchiyama, T., & Yoshiura, K. (2006). A mutation in RYK is a genetic factor for nonsyndromic cleft lip and palate. *The Cleft Palate-craniofacial Journal*, 43, 310–316.
- Weksberg, R., Shuman, C., Caluseriu, O., Smith, A. C., Fei, Y. L., Nishikawa, J., Stockley, T. L., Best, L., Chitayat, D., Olney, A., Ives, E., Schneider, A., Bestor, T. H., Li, M., Sadowski, P., & Squire, J. (2002). Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Human Molecular Genetics*, 11, 1317–1325.
- Wong, A. H., Gottesman, II, & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*, 14 Spec No 1, R11–18.
- Zuccherro, T. M., Cooper, M. E., Maher, B. S., Daack-Hirsch, S., Nepomuceno, B., Ribeiro, L., Caprau, D., Christensen, K., Suzuki, Y., Machida, J., Natsume, N., Yoshiura, K., Vieira, A. R., Orioli, I. M., Castilla, E. E., Moreno, L., Arcos-Burgos, M., Lidral, A. C., Field, L. L., Liu, Y. E., Ray, A., Goldstein, T. H., Schultz, R. E., Shi, M., Johnson, M. K., Kondo, S., Schutte, B. C., Marazita, M. L., & Murray, J. C. (2004). Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *The New England Journal of Medicine*, 351, 769–780.